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Identification and Regulation of antiporters in Strawberry (*Fragaria x ananassa*) under salinity stress

Muhammad SAMEEULLAH[1,2*], Vahdettin ÇİFTÇİ[2]

Highlights:

- Sweet Charlie exhibited a high level of salt tolerance
- Salinity stress increased antiporter gene expression
- *FaSOS1.2*, *FaSOS3*, and *FaNHX1.2* played roles in salt tolerance.

Keywords:

- Salinity
- Antiporters
- Gene regulation
- *SOS*
- *NHX*

ABSTRACT:

Soil salinity is a major abiotic constraint that adversely affects plant growth and yield. The strawberry cultivar Sweet Charlie, previously reported as salt tolerant, was selected to evaluate physiological responses and to identify and characterize the differential regulation of antiporter genes under salinity stress. In total, ten antiporters were identified in cultivated strawberry, belonging to six gene families. The *SOS* (Salt Overly Sensitive) family comprised three members (*FaSOS1.1*, *FaSOS1.2*, and *FaSOS1.3*), while *SOS2*, *SOS3*, *SOS4*, and *SOS5* were each represented by a single gene (*FaSOS2*, *FaSOS3*, *FaSOS4*, and *FaSOS5*). The NHX (cation/H^+ exchanger) family included three members (*FaNHX1.1*, *FaNHX1.2*, and *FaNHX2*). The cultivar Sweet Charlie exhibited a high level of salt tolerance, with an LT_{50} corresponding to 85 mM NaCl after 7 days of stress exposure. Physiological adaptations such as maintenance of leaf area, preservation of relative water content through stomatal regulation, osmotic adjustment, and sustained chlorophyll levels contributed to mitigating the detrimental effects of short-term salinity stress. At the molecular level, increased expression of *FaSOS1.2* and *FaSOS3* in roots, along with *FaNHX1.2* in both roots and shoots, suggests their involvement in alleviating salt-induced toxicity. Short-term salinity stress also induced cellular damage, as evidenced by enhanced reactive oxygen species (ROS) accumulation, elevated malondialdehyde (MDA) levels, and increased electrolyte leakage. Future work will focus on the overexpression of selected upregulated antiporters (*FaSOS1, FaSOS3, and FaNHX1.2*) in *Arabidopsis thaliana* to further elucidate their functional roles in salinity tolerance.

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INTRODUCTION

Salinity is a major global problem that limits land use and crop productivity. More than 20% of irrigated lands are affected by salinity, mainly due to improper agricultural practices. Excess sodium (Na^+) is the major toxic ion in saline soils, interfering with potassium (K^+) uptake and reducing the cytosolic K^+:Na^+ ratio, thereby affecting enzyme activity, metabolism, and photosynthesis. Under saline conditions, plants experience both osmotic stress and Na^+ toxicity. To cope with salinity stress, plants employ several physiological and biochemical mechanisms, including accumulation of compatible solutes such as proline and glycine betaine, and regulation of Na^+ homeostasis to minimize ion toxicity.

Strawberry (*Fragaria* × *ananassa*) is an economically important horticultural crop widely cultivated in Türkiye and other parts of the world. However, strawberry is highly sensitive to salinity, which negatively affects plant growth, fruit quality, and yield through ion toxicity, nutrient imbalance, leaf burn, and growth inhibition. Several studies have demonstrated considerable variation in salinity tolerance among strawberry cultivars. Saied et al. (2005) reported significant reductions in fruit yield and quality in 'Korona' and 'Elsanta' under NaCl stress, with 'Elsanta' being more sensitive. Similarly, Orsini et al. (2012) observed reductions in leaf area and shoot dry weight in 'Elsanta' and 'Elsinore' under saline irrigation. Other studies identified cultivars such as 'Korona', 'Tro', 'Yalova-104', 'Yalova-15', 'Yalova-16', 'Arnavutköy', 'Camarosa', and 'Albion' as relatively more tolerant to salinity (Turhan and Eris 2009; Ferreira et al. 2014). Previous reports also indicated that 'Sweet Charlie' exhibits better biomass production and lower sodium accumulation under salt stress compared with sensitive cultivars such as 'Chandler'. These findings highlight the substantial variability in salinity tolerance among strawberry cultivars and the importance of selecting suitable genotypes for saline environments.

By the employment of forward and reverse genetics five salt tolerant genes have been found in Arabidopsis namely *SOS1* (Wu et al. 1996), *SOS2* (Zhu et al. 1998), *SOS3* (Liu and Zhu 1997), *SOS4* (Shi et al. 2002), and SOS5 (Shi et al. 2003).

SOS1 encodes a protein that has sequence similarity with mammalian and yeast Na^+/H^+ antiporters (Shi et al. 2000). *SOS2* encodes a serine/threonine protein kinase that contains two functional domains: an N-terminal catalytic domain similar to that of the yeast SNF1 kinase and a novel C-terminal regulatory domain (Liu et al. 2000). *SOS3* was the first cloned *SOS* gene and it encodes a protein with three predicted EF-hands for Ca2+ binding (Liu and Zhu 1998). *SOS4* involved in biosynthesis of pyridoxal-5-phosphate (PLP), an active form of vitamin B6 (Shi and Zhu 2002). *SOS5* encodes a fasciclin-like arabinogalactan protein that is important for cell wall formation in plants (Shi et al. 2003). Mutation in the *SOS5* gene results in root tip swelling and arrested root growth under salt stress. Yeast complementation assays identified a yeast *NHX1* homologue, *AtNHX1*, as complementing the yeast *nhx1* mutant (Gaxiola et al. 1999). *AtNHX1* confers salt tolerance to yeast cells by sequestering Na^+ into the vacuole driven by the H^+ gradient across the vacuolar membrane. The Na^+/H^+ exchange mediated by *AtNHX1* is electroneutral and sensitive to amiloride (Quintero et al. 2000; Darley et al. 2000). The expression of *AtNHX1* is up-regulated by salt stress and ABA, which suggests the importance of *AtNHX1* in salt tolerance in Arabidopsis (Gaxiola et al. 1999; Quintero et al. 2000; Shi and Zhu 2002). The important role of *AtNHX1* in salt tolerance in plants was suggested by overexpression of *AtNHX1* in different plant species conferring salt tolerance to transgenic plants (Apse et al. 1999; Zhang and Blumwald 2001; Zhang et al. 2001). These studies indicate the potential role of antiporters from different gene families to ameliorate salinity toxicity in glycophytes.

This study aimed to identify and characterize the regulation of antiporters in strawberry plants under salinity stress, as well as to investigate the physiological responses of the 'Sweet Charlie' cultivar.

MATERIALS AND METHODS

Plant material and growth conditions

A pot experiment conducted with strawberry (*Fragaria x ananassa* cv. Sweet Charlie). Frigo strawberry seedlings were planted in pots containing mixture of peat and vermiculite (3:1). The plants with well-developed root system were washed and transferred to hydroponic system. The modified 1/2 strength of Hoagland nutrient solution aerated continuously (Hoagland and Arnon 1950). The pH of the nutrient solution adjusted to 5.8-6.0 using 1N KOH. The growth room had 16/8 light/dark cycle (250 $\mu mol\ m^{-2}\ s^{-1}$) with temperatures of between 23-28 ℃ and a 65% relative humidity. The nutrient solutions renewed every week. Three weeks after planting, NaCl treatments initiated by adding 0, 25 and 50 for MDA content or 0, 50 and 100, 150 and 200 mM NaCl into the nutrient solution (day 0) for 7 days for electrical conductivity measurement and cell membrane stability in order to determine LT50. LT_{50} was defined as the salt concentration causing 50% injury to the plants (Arora et al. 1992). All measurements were performed with three biological replicates.

Plant growth conditions for antiporters regulation and expression

Strawberry plants were treated with suitable 85 mM NaCl dose as mentioned above under plant material and growth conditions.

These seedlings were grown hydroponically in 1/2 Hogland solution following the procedure described above. The seedlings were treated without (control) or with NaCl treatment (85 mM). Tissues (roots and shoots) were harvested after 7 days and immediately frozen in liquid nitrogen and then stored at -80˚C for RNA extraction.

Determination electrolyte leakage to assess cell membrane stability

Electrolyte leakage was measured to assess membrane permeability following Arora et al. (1992) with slight modifications. After one week of salinity treatment (0, 50, 100, and 200 mM NaCl), fully expanded leaves were collected, and 2 cm leaf discs were incubated in 20 mL distilled water after vacuum infiltration. Initial electrical conductivity (C1) was measured after shaking samples for 4 h at room temperature. Samples were then autoclaved at 121°C for 15 min, cooled, and final conductivity (C2) was recorded. Electrolyte leakage was calculated as % electrolyte leakage = (C1/C2) × 100. Percentage injury and LT_{50} values were determined according to Arora et al. (1992). All analyses were performed with three biological replicates.

Chlorophyll measurement

Chlorophyll a, b and total contents were determined by following the procedure as described previously (Altuğ et al. 2019).

In silico based Identification of putative strawberry antiporters

Arabidopsis antiporters and has been classified by Ward (2001) in detail. Following Arabidopsis antiporter gene families, the genes which highly expressed based on microarray under salinity stress either in root or shoot were selected as probe to identify strawberry salinity tolerant genes. The protein sequences of Arabidopsis antiporters were blast against strawberry genome to retrieve putative strawberry antiporters.

Phylogenetic and classification of strawberry antiporters

Putative strawberry antiporters were aligned with Arabidopsis antiporters using MUSCLE online tool. Aligned multiple sequences were used to build neighbor joining based phylogenetic tree with 1000 replications and using partial deletion using MEGA 6.0.

RNA extraction, cDNA synthesis and qRT-PCR based expression of putative antiporter

The procedure was followed as described earlier with modifications (Sameeullah et al. 2013). Briefly, total RNA isolated from the plant tissues (approximately 100 mg) after the treatments using Plant RNA Isolation Aid (Life Technologies, Carlsbad, CA, USA) kit and then treated with RNase-free DNaseI (Thermoscientific, USA) to remove genomic DNA contamination.

RNA quantified using Quantus Fluorometer (Promega, USA). cDNA synthesized by iScript cDNA Synthesis Kit (Hercules, California, USA) from 1 µg total RNA. Real time PCR reaction mixture and prepared according to iTaq Universal SYBR Green Supermix SMX200 (Hercules, California, USA) instructions. For each reaction, 15 µL total reaction mixture prepared. The CFX96 Real time PCR system (Hercules, California, USA) used to detect gene expression. House keeping gene *Fa-GAPDH2* used to normalize the gene expression (Khan and Shih 2004).

Table 1. List of primers to determine expression of antiporters by qRT-PCR

Gene name	Primer sequence 5'-3'	Product length (bP)	Reference
FaNHX1 F	5'- GGGCAGCATTCATATTCCCG -3'	201	This study
FaNHX1 R	5'- TGGAGGTGATCATCAGTGCA -3'		
FaNHX1.2 F	5'- AGGTGGACTTCAATGGAGGG -3'	172	This study
FaNHX1.2 R	5'- TCAGAGCCGAAGTTGAGTGT -3'		
FaNHX2 F	5'- CCTCCACTCACCATCAACCT -3'	192	This study
FaNHX2 R	5'- CGATGGATGAGCAATGGGTG -3'		
FaSOS1 F	5'-AGGCGTCCAAGAAGAACTGA-3'	150	This study
FaSOS1 R	5'-CTGGATCCTTGATGGGAAAA-3'		
FaSOS1.2 F	GCCTTTCTTCGTGCCCATAG	170	This study
FaSOS1.2 R	GTTTTCACAACGCGCAGAAC		
FaSOS1.3 F	GAAGAGGACCGCTCGAGTAC	162	This study
FaSOS1.3 R	ATCAATAGCTGCCCCTACGG		
FaSOS2 F	5'- TCGTGTAAACCGGTTGAAGG -3'	224	This study
FaSOS2 R	5'- CTGCTAAATTAGACCGCCGG -3'		
FaSOS3 F	5'- GCGTACTTCACTGGTCCAAC -3'	188	This study
FaSOS3 R	5'- GCCAAGCCTCCACAAAGTAG -3'		
FaSOS4 F	5'- CCTTTGGCTGAGCGTTTAGG -3'	222	This study
FaSOS4 R	5'- CCCACCACTGAAGAATGCAC -3'		
FaCHX5 F	5'- TGTTGTCTTCGCTGGTGTTG -3'	183	This study
FaCHX5 R	5'- TGTTGCCGGCATTTTCTTCA -3'		
Fv-GAPDH2F	5'- GAGTCTACTGGAGTGTTCA-3'	135	Topçu (2022)
Fv-GAPDH2 R	5'-CCTGTATTCGTGCTCATTCA-3'		

Histochemical detection of $O2^{\cdot-}$ and H_2O_2 in leaves

Histochemical localization of superoxide radicals ($O_2^{\cdot-}$) in leaves was performed according to Fryer et al. (2002) and Zhou et al. (2008) using nitro blue tetrazolium (NBT) staining. Leaves were immersed in 0.1% (w/v) NBT solution containing 10 mM sodium azide and 50 mM potassium phosphate buffer (pH 6.4), followed by vacuum infiltration (~100–150 mbar for 1 min). After incubation for 20 min under cool fluorescent light at room temperature, chlorophyll was removed with 95% ethanol. Blue coloration indicated the presence of $O_2^{\cdot-}$.

Histochemical detection of H_2O_2 was carried out using 3,3′-diaminobenzidine (DAB) staining according to Xue et al. (2009). Leaves were infiltrated with 1 mg mL^{-1} DAB solution (pH 3.8) under

vacuum and then boiled in 95% ethanol for 10 min to remove chlorophyll. Formation of dark brown coloration indicated H_2O_2 accumulation, and stained leaves were photographed for documentation.

Stomatal aperture measurement

Strawberry leaves were harvested after treatment with NaCl (85 mM) with or without treatment (CK). Stomata aperture opening and closing was observed under microscope (Leica DM1000 LED, Switzerland) and photographed were taken. The length and width of stomata aperture was calculated using Leica Application Suit (LAS EZ) microscope software.

Leaf Area

The leaf area was measured with ImageJ analysis software (Schneider et al. 2012), in six plants of each treatment. For the measurement of leaf area, three expanded leaves, each with three leaflets was selected.

Determination of Leaf Osmotic Potential (Ψs)

The osmotic potential was determined as described previously (Faralli et al. 2015). The harvested leaves were immediately frozen in liquid nitrogen and left to room temperature to be thawed. The extracted juice from the disintegrated cells was transferred to the "osmometer" (Wescor Vapor Pressure Osmometer 5600) with the aid of a pipette and the osmotic potential (Ψπ) measurement was performed. The osmolarity values were converted from mozmol kg^{-1} to MPa using Van't Hoff equation using the formula:

$\Psi\pi$ (MPa) = −c (mosmoles kg^{-1}) × 2.58 × 10−3 according to the Van't Hoff equation.

Water Loss Determination

Leaf relative water content (RWC) will be calculated according to Yamasaki and Dillemburg (1999). Fully expanded leaves will be weighed to obtain fresh weight (FW). The leaves will be floated on distilled water in closed petri dishes for 3 h and then their turgid weights (TW) will be measured. The leaves will be dried at 80 °C for 48 h in a draft-oven to determine dry weight (DW). Leaf RWC was calculated by using the following formula:

Leaf RWC = FW-DW/TW-DW*100

Lipid peroxidation

Lipid peroxidation was determined by measuring malondialdehyde (MDA) content according to Heath and Packer (1968).

Statistical method

The replicated data were recorded, and the mean values were calculated and plotted as graphs with mean ± SE using Microsoft Excel.

RESULTS AND DISCUSSION

Determination electrolyte leakage to assess cell membrane stability and chlorophyll

The cell membrane injury level enhanced in strawberry leaves with the increment in salinity level as shown in Figure 1A. Cell membrane injury level under varying level of salinity was used to determine LT_{50} where 50% reduction in plant growth was monitored (Figure 1B).

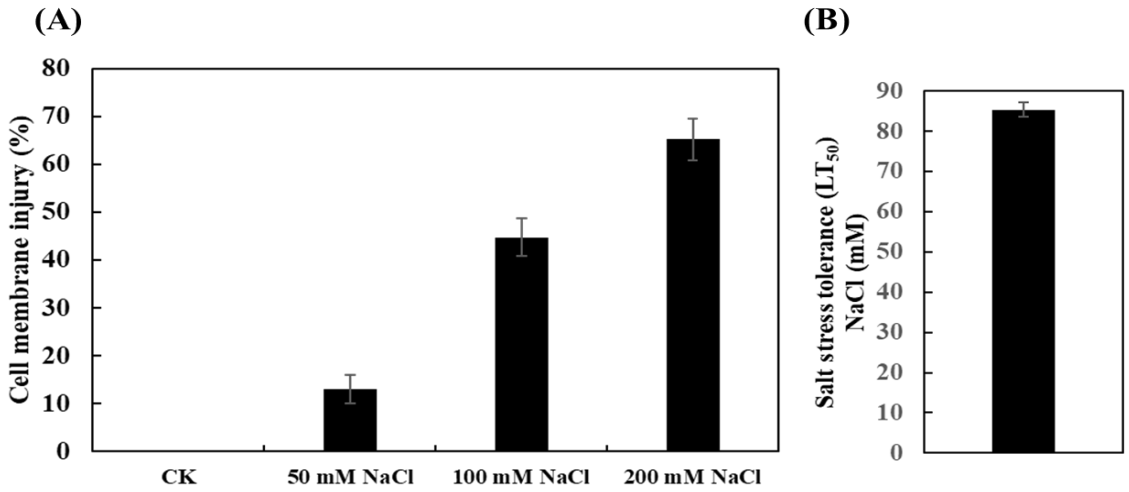

Figure 1. Cell membrane injury and salt tolerance index salinity stress. Salt tolerance index measured based on cell membrane injury level. 85 mM NaCl caused 50% reduction in Sweet Charlie over 1 week. Data represents mean ± SE of three repeats

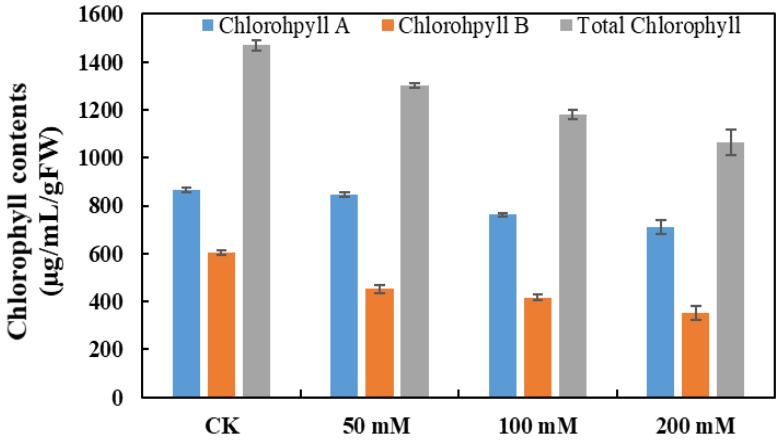

Figure 2. Chlorophyll contents of strawberry leaves under various level of salinity. Data represents mean ± SE of three repeats.

Chlorophyll contents were decreased with increasing level of salinity over one-week period of time as shown in Figure 2. Although the drastic effect of salinity on chlorophyll was not so prominent due to short period of salinity stress in hydroponic culture containing ½ strength Hoagland solution. These results show that Sweet Charlie is relatively salt tolerant cultivar compared to other cultivars camarosa and chandler (Turhan et al. 2008) which show EC50 below 50 mM NaCl. However, Sweet Charlie cultivar exhibited EC50 at 85 mM NaCl.

In silico identification of antiporters

Protein sequences of *SOS1*, *SOS2*, *SOS3*, *SOS4*, *SOS5* and *NHX* genes were retrieved from Arabidopsis resource database (Berardini et al. 2015). Using the Arabidopsis protein sequences as bait in Garden strawberry genome database for *Fragaria x ananassa* (Hirakawa et al. 2014), strawberry antiporter homologues to Arabidopsis were retrieved. Protein sequences of strawberry antiporters were used to construct phylogenetic tree following neighbor joining method. The cDNA sequences of strawberry antiporters were used to design primers for gene expression study. Phylogenetic tree of strawberry antiporters shown in Figure 3. Phylogenetic analysis revealed that the identified strawberry antiporters were clearly grouped into five major clusters corresponding to the NHX, SOS1, SOS2, SOS3,

SOS4, and SOS5 families (Figure 3). The Fragaria × ananassa (Fa) antiporters clustered closely with their homologs from *Fragaria vesca* (Fv) and *Arabidopsis thaliana* (At), indicating a high degree of evolutionary conservation among these transporters. Members of the NHX family, including FaNHX1.1, FaNHX1.2, and FaNHX2, formed a distinct cluster together with AtNHX1/2 and FvNHX1/2, suggesting functional conservation in Na^+/H^+ exchange and ion homeostasis under salinity stress. Similarly, FaSOS1.1, FaSOS1.2, and FaSOS1.3 clustered with FvSOS1 and AtSOS1, supporting their probable involvement in sodium transport and salt stress responses. The close association of FaSOS2, FaSOS3, FaSOS4, and FaSOS5 with their corresponding homologs further indicates conserved evolutionary relationships and potential functional similarity among strawberry and Arabidopsis antiporter-related genes.

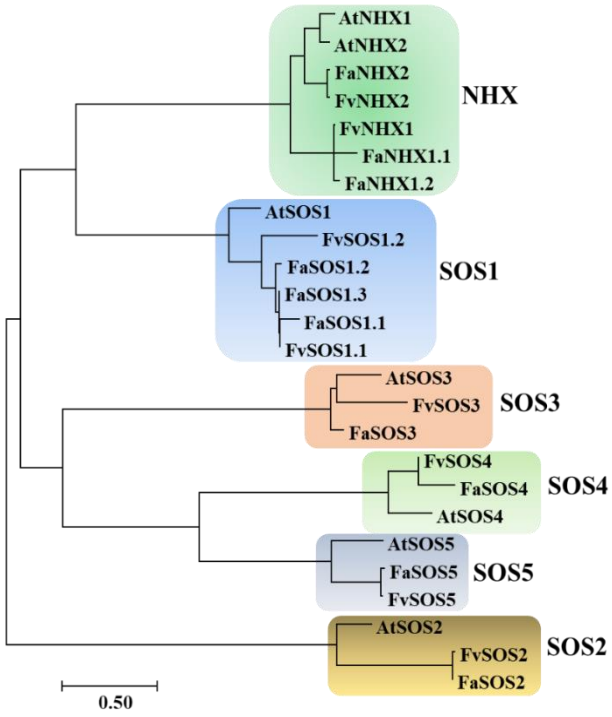

Figure 3. Phylogenetic tree of Strawberry antiporter gene families. Strawberry antiporters belong to six gene families viz. *NHX* (Cation/H^+ exchanger), *SOS1* (SALT OVERLY SENSITIVE 1), *SOS2* (SALT OVERLY SENSITIVE 2), *SOS3* (SALT OVERLY SENSITIVE 3), *SOS4* (SALT OVERLY SENSITIVE 4) and *SOS5* (SALT OVERLY SENSITIVE 5).

Differential expression of strawberry antiporters under salinity stress

The expression profiles of *FaSOS* and *FaNHX* genes showed differential responses to 85 mM NaCl treatment in strawberry (Figure 4A–B). Among the *FaSOS* family members, *FaSOS1.2* exhibited the strongest induction, particularly in root tissues, where its expression increased markedly under salinity stress. Similarly, *FaSOS3* was also highly upregulated in roots under salt treatment, while *FaSOS1.3* showed relatively higher expression in shoots. In contrast, *FaSOS1.1*, *FaSOS4*, and *FaSOS5* displayed only minor changes in expression under salinity conditions. *FaSOS2* showed moderate induction in roots but decreased expression in shoots under salt stress. Among the *FaNHX* genes, *FaNHX1.2* showed the highest expression under salinity stress in both shoots and roots, whereas *FaNHX1.1* was only slightly induced. In contrast, *FaNHX2* expression remained stable in shoots but decreased in roots under NaCl treatment. These results suggest that *FaSOS1.2*, *FaSOS3*, and *FaNHX1.2* may play important roles in Na^+ homeostasis and salt tolerance in strawberry during short-term salinity

stress. Previous studies reported that overexpression of multiple salt-responsive genes can improve salinity tolerance in plants. Yang et al. (2009) reported that overexpression of antiporters in Arabidopsis plants exhibited enhanced salt tolerance comparable to plants overexpressing either *SOS1* or *SOS3* alone. In agreement with these findings, the strong induction of *FaSOS1.2* and *FaSOS3* in roots observed in the present study suggests their possible involvement in reducing Na^+ toxicity under saline conditions. The *AtNHX1* gene encodes a vacuolar Na^+/H^+ antiporter involved in the transport of both K^+ and Na^+ into vacuoles (Apse et al. 1999; Zhang and Blumwald 2001). Therefore, the elevated expression of *FaNHX1.2* in both shoots and roots indicates that this gene may contribute to Na+ sequestration and salinity tolerance in strawberry. Future studies involving overexpression of *FaSOS1.2*, *FaSOS3*, and *FaNHX1.2* in transgenic plants may further clarify their roles in salt stress adaptation.

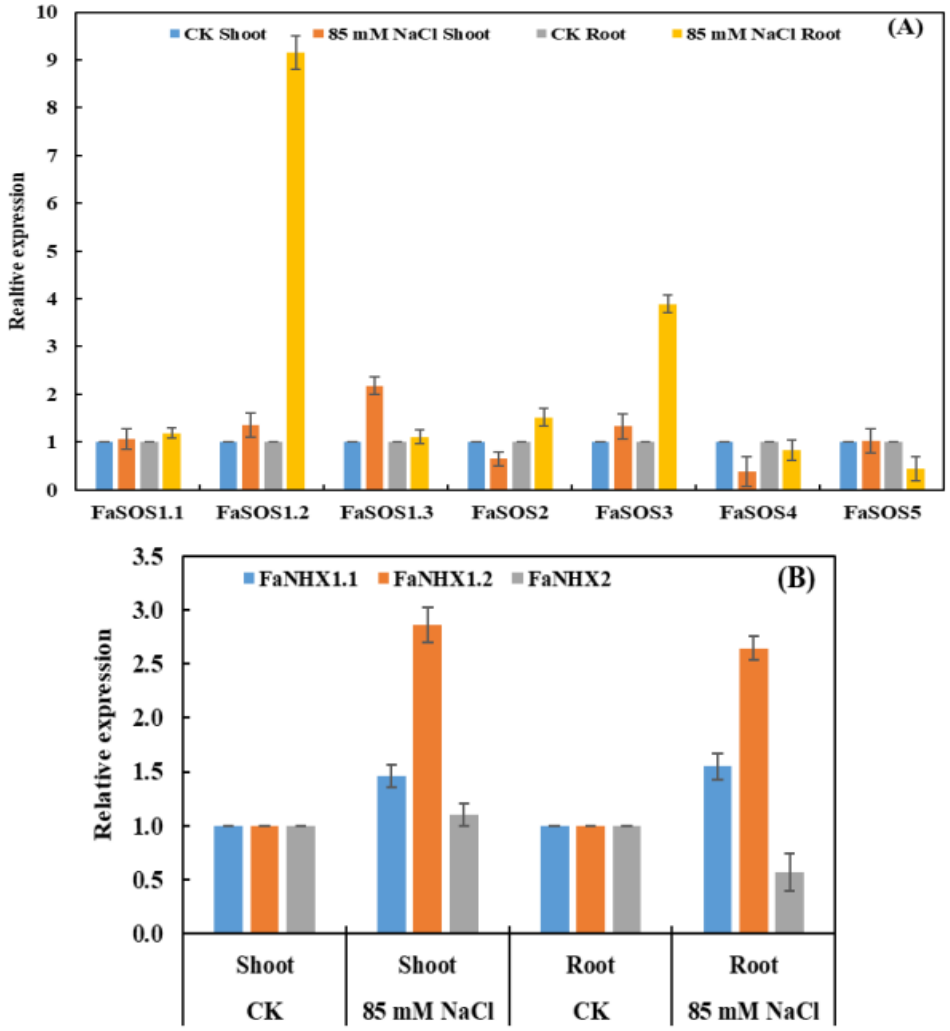

Figure 4. Differential expression of antiporters in shoot and root tissues under 85 mM NaCl over 7 days. Data represent mean ± SE of three biological repeats each with three technical repeats.

Histochemical detection of $O_2^{\bullet -}$ and H_2O_2 in leaves

Histochemical detection of $O_2^{\bullet -}$ (super oxide) and H_2O_2 (hydrogen peroxide) was observed in 7 days treated CK and salt stressed plant leaves using NBT and DAB. Upper panel of leaf discs were treated with DAB to detect H_2O_2. In CK and salt stressed plants. CK leaf showed less DAB staining compared to 85 mM NaCl treated plants. Under salt stress higher amount of H2O2 produced due to salinity therefore dark brownish color observed in Figure 5A. NBT staining was performed to detect production of superoxide (O_2^-) in salt stressed plant leaves. Dark blue staining was observed in salt

stressed plant leaf than in CK plant leaf indicate the production of superoxide due to salinity Figure 5B. Numerous stresses induces the formation of reactive oxygen species (Pitzschke et al. 2006; Miller et al. 2008). The damage of cellular components caused by ROS production can be inhibited to some extent through the action of ROS scavenging enzymes. Superoxide (O_2^-) is particularly aggressive. The dark blue staining indicates provoked enhanced ROS in salt stress plant leaf suggest that Sweet Charlie may not able to exclude toxic effect of salinity in leaf as shown in DAB and NBT staining leaves. However, on the other hand the highest expression of antiporters in root tissue suggest that might be upregulated antiporters *FaSOS1.2*, *FaSOS3* and *FaNHX1.2* could exclude or prevent influx of Na^+ into the root tissue to some extent for the short period of salinity stress.

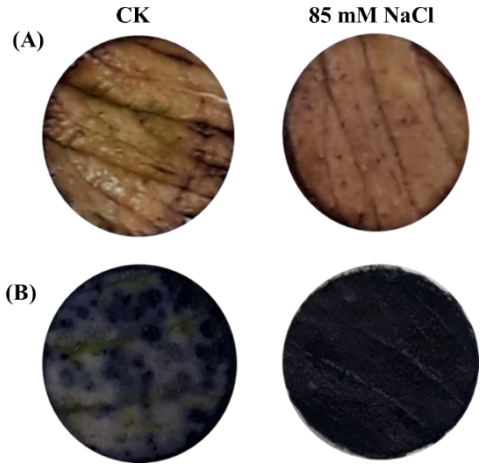

Figure 5. Histochemical detection of superoxide and hydrogen peroxide in strawberry leaves

Stomatal aperture measurement

Stomata aperture was observed in CK and salt stressed (85 mM NaCl) plant leaves using epidermal cells of the lower surface of the leaf. Width of CK stomata aperture was higher than salt stressed leaves with negligible or no opening was observed (Figure 6, upper panel). However, length of stomata of both CK and salt stressed leaves were same. Lower panel represent the stomata opening and closing of CK and salt stressed plants over 7 days (Figure 6). Under moderate stress conditions, a slight reduction in stomatal conductance can serve as an adaptive response by minimizing water loss and enhancing water-use efficiency in plants. Both drought and salinity stress stimulate the synthesis of abscisic acid (ABA) in roots, which is subsequently transported to aerial tissues where it promotes stomatal closure and limits cell expansion and growth. Turhan et al. (2008) reported that NaCl treatment rapidly decreased stomatal conductance (g_s) in the salt-sensitive cultivar 'Chandler', while no significant changes in g_s were observed in the more tolerant cultivars 'Tioga' and 'Camarosa' at salinity levels up to 34.0 mM NaCl. These findings suggest that stomatal closure may function as an early signaling mechanism that initiates various stress-response pathways, including growth suppression, which can ultimately negatively affect crop yield (Awang and Atherton 1995; Maggio et al. 2002; Ruggiero et al. 2004).

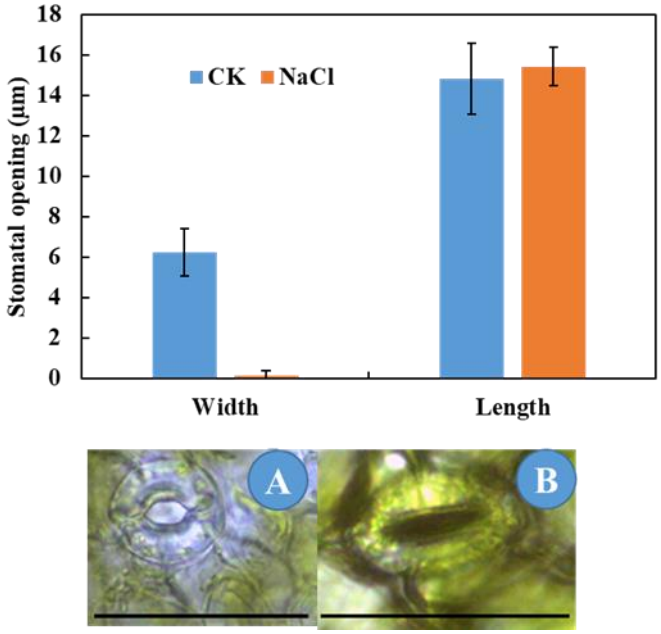

Figure 6. Stomata behavior of strawberry under salt stress (85 mM NaCl) for 7 days treatment. Data represent mean ± SE observations of 15 samples from CK and salt stressed leaves. Lower panel are representative of above bar graph data. (A) stomata opening of control leaf, (B) stomata closure of salt stressed leaf. Scale bar shows 50 µm

However, salt treatment presented by Turhan et al. (2008) were lower level of NaCl concentrations (maximum 34 mM) compared to in this study where salinity level was 85 mM. The chlorophyll content, electrolyte leakage and higher level of LT50 show that Sweet Charlie is more salt tolerant cultivar than the Camarosa, Chandler and Tioga.

Physical attributes of control and salt stressed plants

The leaf area, relative water content, dry weight, and osmotic potential were measured in control (CK) and salt-stressed (85 mM NaCl) plants. Over 7 days of treatment, no remarkable difference was observed in leaf area between CK and salt-treated plants (Figure 7A). Similarly, relative water content remained at comparable levels in both control and salt-stressed plants (Figure 7B). However, a clear reduction in dry weight (DW) was observed in salt-stressed plants compared with CK plants after 1 week of treatment (Figure 7C). Osmotic potential also remained at similar levels between CK and salt-treated plants during the short-term stress period (Figure 7D). These results indicate that short-term salinity treatment had no substantial negative effects on leaf area, relative water content, or osmotic potential, suggesting that the cultivar maintained its physiological attributes to sustain growth under salt stress. However, prolonged salinity treatment negatively affected plant dry weight, indicating that long-term exposure to high salinity exerts more detrimental effects than short-term stress

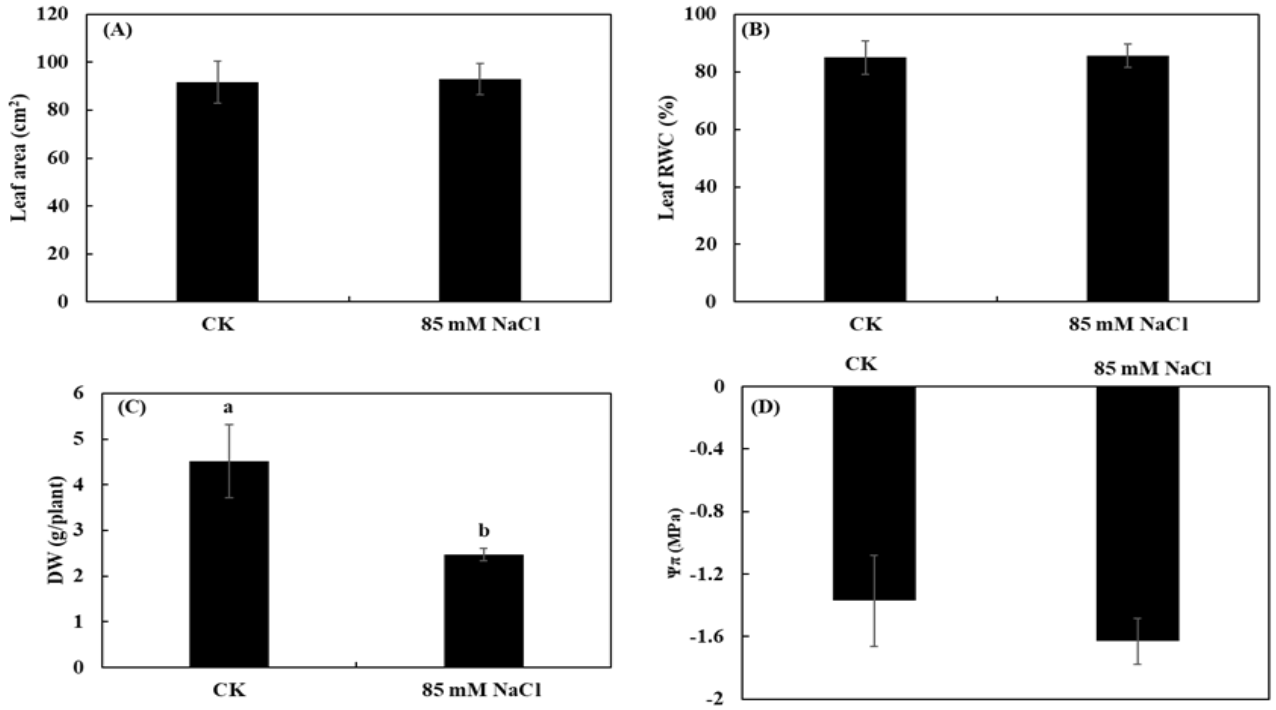

Figure 7. Determination of leaf area, leaf relative water content and osmotic potential and dry weight over 7 days under control and salt stress (85 mM NaCl). Data represent mean ± SE of three repeats.

Lipid peroxidation

Lipid peroxidation, as an indicator of cellular damage determined as the level of malondialdehyde (MDA) in plants under the stress. MDA contents measured after 1 week of treatment and the contents increased with increasing level of salinity (Figure 8 A-B). The toxic effect of salt is attributable to a large degree on the induction of oxidative stress. Relative tolerance of plants to the salinity stress was assessed by measuring different parameters viz., RWC, electrolyte leakage, accumulation of MDA, O^{2-}, and H_2O_2. Maintenance of high water status, expressed as RWC, leaf area and osmotic potential is an easily assessable indicator of stress tolerance for short period of stress.

The increased electrolyte leakage under increased salinity level suggested much severe membrane damage in plants at the higher concentration of salt. Similarly, higher MDA content were observed at the highest salinity level. MDA accumulates as a result of lipid peroxidation and is an effective indicator of oxidative damage (Yoshimura et al. 2004). The production of ROS (as hydrogen peroxide and superoxide), electrolyte leakage, and MDA content indicate that at cellular level these attributes contribute to damage at cellular level however at phenotypic level such as leaf area and maintenance of chlorophyll content was unaffected beside osmotic potential and relative water content.

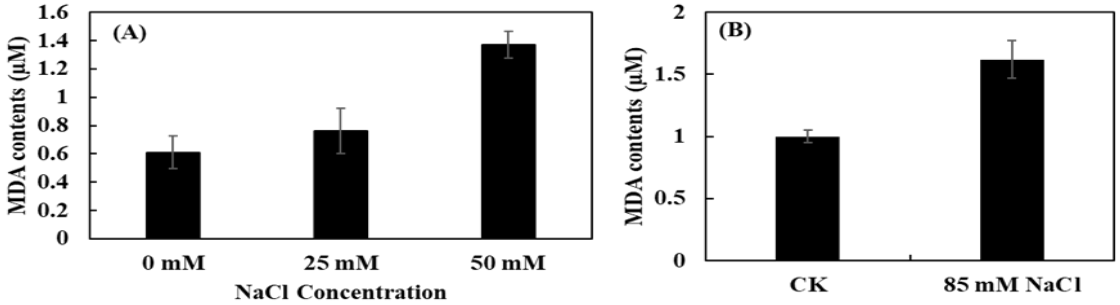

Figure 8. MDA content in CK and salt stressed strawberry plants. Data represent mean ± SE of three replicates.

CONCLUSION

The strawberry cultivar Sweet Charlie was evaluated for several physiological and biochemical attributes, including electrolyte leakage, salt tolerance index, chlorophyll content, leaf area, relative water content, osmotic potential, dry weight, stomatal aperture size, and cellular damage under control and salinity stress conditions. In addition, antiporters belonging to six different gene families were identified, and their differential expression was analyzed in roots and shoots of control and salt-stressed plants. The expression analysis revealed that *FaSOS1.2*, *FaSOS3*, and *FaNHX1.2* were strongly induced under salinity stress, suggesting that these antiporters may play important roles in protecting strawberry plants against salt stress through maintenance of ion homeostasis and cellular tolerance mechanisms.

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Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

REFERENCES

Altuğ, C., Saleh, L. Y., Caner, E., Güneş, H., & Sameeullah, M. (2019). Multicomponent synthesis of novel thiazolo[3,2-a]pyridin-8-yl-phosphonates as a model of plant growth regulator. Phosphorus, Sulfur, and Silicon and the Related Elements, 194(1–2), 111–119.

Apse, M. P., Aharon, G. S., Snedden, W. A., & Blumwald, E. (1999). Salt tolerance conferred by overexpression of a vacuolar Na+/H+ antiport in Arabidopsis. Science, 285(5431), 1256–1258.

Arora, R., Wisniewski, M. E., & Scorza, R. (1992). Cold acclimation in genetically related (sibling) deciduous and evergreen peach (Prunus persica [L.] Batsch). I. Seasonal changes in cold hardiness and polypeptides of bark and xylem tissues. Plant Physiology, 99(4), 1562–1568.

Hoagland, D. R., & Arnon, D. I. (1950). The water-culture method for growing plants without soil. Circular 347, University of California Agricultural Experiment Station, Berkeley.

Awang, Y. B., & Atherton, J. (1995). Growth and fruiting responses of strawberry plants grown on rockwool to shading and salinity. Scientia Horticulturae, 62(1–2), 25–31.

Barroso, M. M., & Alvarez, C. (1997). Toxicity symptoms and tolerance of strawberry to salinity in the irrigation water. Scientia Horticulturae, 71(3–4), 177–188.

Berardini, T. Z., Reiser, L., Li, D., Mezheritsky, Y., Muller, R., Strait, E., & Huala, E. (2015). The Arabidopsis information resource: Making and mining the "gold standard" annotated reference plant genome. Genesis, 53(8), 474–485.

Bates, L. S., Waldren, R. P., & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. Plant and Soil, 39, 205–207.

Carvajal, M., Martinez, V., & Cerda, A. (1999). Influence of magnesium and salinity on tomato plants grown in hydroponic culture. Journal of Plant Nutrition, 22, 177–190.

Darley, C. P., Van Wuytswinkel, O. C., Van Der Woude, K., & Mager, W. H. (2000). Arabidopsis thaliana and Saccharomyces cerevisiae NHX1 genes encode amiloride sensitive electroneutral Na+/H+ exchangers. Biochemical Journal, 351(1), 241–249.

Díaz-López, L., Gimeno, V., Lidón, V., Simón, I., Martínez, V., & García-Sánchez, F. (2012). The tolerance of Jatropha curcas seedlings to NaCl: An ecophysiological analysis. Plant Physiology and Biochemistry, 54, 34–42.

Faralli, M., Lektemur, C., Rosellini, D., & Gürel, F. (2015). Effects of heat shock and salinity on barley growth and stress-related gene transcription. Biologia Plantarum, 59(3), 537–546.

Ferreira, J. F. S., Liu, X., & Suarez, D. L. (2014). Salinity tolerance of five commercial cultivars of strawberry (Fragaria × ananassa Duch.). In Proceedings of the 3rd International Salinity Forum (pp. 170–171), Riverside Convention Center, CA, USA.

Fryer, M. J., Oxborough, K., Mullineaux, P. M., & Baker, N. R. (2002). Imaging of photo-oxidative stress responses in leaves. Journal of Experimental Botany, 53(372), 1249–1254.

Gaxiola, R. A., Rao, R., Sherman, A., Grisafi, P., Alper, S. L., & Fink, G. R. (1999). The Arabidopsis thaliana proton transporters, AtNhx1 and Avp1, can function in cation detoxification in yeast. Proceedings of the National Academy of Sciences, 96(4), 1480–1485.

Heath, R., & Packer, L. (1968). Photooxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics, 125, 189–198.

Hirakawa, H., Shirasawa, K., Kosugi, S., Tashiro, K., Nakayama, S., Yamada, M., Kohara, M., Watanabe, A., Kishida, Y., & Fujishiro, T. (2014). Dissection of the octoploid strawberry genome by deep sequencing of the genomes of Fragaria species. DNA Research, 21(2), 169–181.

Husaini, A. M., & Abdin, M. Z. (2008). Development of transgenic strawberry (Fragaria × ananassa Duch.) plants tolerant to salt stress. Plant Science, 174, 446–455.

Kepenek, K., & Koyuncu, F. (2000). Studies on the salt tolerance of some strawberry cultivars under glasshouse conditions. In International Symposium on Techniques to Control Salination for Horticultural Productivity 573 (pp. 297–304).

Keutgen, A. J., & Pawelzik, E. (2008). Quality and nutritional value of strawberry fruit under long term salt stress. Food Chemistry, 107(4), 1413–1420.

Liu, J., Ishitani, M., Halfter, U., Kim, C. S., & Zhu, J. K. (2000). The Arabidopsis thaliana SOS2 gene encodes a protein kinase that is required for salt tolerance. Proceedings of the National Academy of Sciences, 97(7), 3730–3734.

Liu, J., & Zhu, J. K. (1997). An Arabidopsis mutant that requires increased calcium for potassium nutrition and salt tolerance. Proceedings of the National Academy of Sciences, 94(26), 14960–14964.

Liu, J., & Zhu, J. K. (1998). A calcium sensor homolog required for plant salt tolerance. Science, 280(5371), 1943–1945.

Loreto, F., & Velikova, V. (2001). Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. Plant Physiology, 127, 1781–1787.

Lutts, S., Kinet, J. M., & Bouharmont, J. (1995). Changes in plant response to NaCl during development of rice (Oryza sativa L.) varieties differing in salinity resistance. Journal of Experimental Botany, 46, 1843–1852.

Maas, E. V., & Hoffman, G. J. (1977). Crop salt tolerance-current assessment. Journal of the Irrigation and Drainage Division, 103(2), 115–134.

Maggio, A., Miyazaki, S., Veronese, P., Fujita, T., Ibeas, J. I., Damsz, B., Narasimhan, M. L., Hasegawa, P. M., Joly, R. J., & Bressan, R. A. (2002). Does proline accumulation play an active role in stress-induced growth reduction? The Plant Journal, 31(6), 699–712.

Miller, G., Shulaev, V., & Mittler, R. (2008). Reactive oxygen signaling and abiotic stress. Physiologia Plantarum, 133(3), 481–489.

Orsini, F., Alnayef, M., Bona, S., Maggio, A., & Gianquinto, G. (2012). Low stomatal density and reduced transpiration facilitate strawberry adaptation to salinity. Environmental and Experimental Botany, 81, 1–10.

Pitzschke, A., Forzani, C., & Hirt, H. (2006). Reactive oxygen species signaling in plants. Antioxidants and Redox Signaling, 8(9–10), 1757–1764.

Quintero, F. J., Blatt, M. R., & Pardo, J. M. (2000). Functional conservation between yeast and plant endosomal Na+/H+ antiporters. FEBS Letters, 471(2–3), 224–228.

Ruggiero, B., Koiwa, H., Manabe, Y., Quist, T., Inan, G., & Saccardo, F. (2004). Uncoupling the effects of ABA on plant growth and water relations: Analysis of sto1/nced3, an ABA-deficient but salt-tolerant mutant in Arabidopsis. Plant Physiology, 136, 3134–3147.

Saied, A. S., Keutgen, A. J., & Noga, G. (2005). The influence of NaCl salinity on growth, yield and fruit quality of strawberry cvs. 'Elsanta' and 'Korona'. Scientia Horticulturae, 103(3), 289–303.

Sameeullah, M., Sasaki, T., & Yamamoto, Y. (2013). Sucrose transporter NtSUT1 confers aluminum tolerance on cultured cells of tobacco (Nicotiana tabacum L.). Soil Science and Plant Nutrition, 59, 756–770. https://doi.org/10.1080/00380768.2013.830230

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. Nature Methods, 9(7), 671–675.

Shi, H., Ishitani, M., Kim, C., & Zhu, J. K. (2000). The Arabidopsis thaliana salt tolerance gene SOS1 encodes a putative Na+/H+ antiporter. Proceedings of the National Academy of Sciences, 97(12), 6896–6901.

Shi, H., Kim, Y., Guo, Y., Stevenson, B., & Zhu, J. K. (2003). The Arabidopsis SOS5 locus encodes a putative cell surface adhesion protein and is required for normal cell expansion. The Plant Cell, 15(1), 19–32.

Shi, H., Xiong, L., Stevenson, B., Lu, T., & Zhu, J. K. (2002). The Arabidopsis salt overly sensitive 4 mutants uncover a critical role for vitamin B6 in plant salt tolerance. The Plant Cell, 14(3), 575–588.

Shi, H., & Zhu, J. K. (2002). SOS4, a pyridoxal kinase gene, is required for root hair development in Arabidopsis. Plant Physiology, 129(2), 585–593.

Steppuhn, H., van Genuchten, M. T., & Grieve, C. M. (2005). Root-zone salinity: I. Selecting a product-yield index and response function for crop tolerance. Crop Science, 45(1), 209–220.

Topçu, H. (2022). Reference gene selection for RT-qPCR normalization of strawberry at various organs, different growing stages of fruits, and salt-stress treatment. *Turkish Journal of Agriculture and Forestry, 46*(4), 517–529. https://doi.org/10.55730/1300-011X.3022

Turhan, E., & Eris, A. (2009). Changes of growth, amino acids, and ionic composition in strawberry plants under salt stress conditions. Communications in Soil Science and Plant Analysis, 40(21–22), 3308–3322.

Turhan, E., Gulen, H., & Eris, A. (2008). The activity of antioxidative enzymes in three strawberry cultivars related to salt-stress tolerance. Acta Physiologiae Plantarum, 30(2), 201–208.

Velikova, V., Yordanov, I., & Edreva, A. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. Plant Science, 151, 59–66.

Ward, J. M. (2001). Identification of novel families of membrane proteins from the model plant Arabidopsis thaliana. Bioinformatics, 17(6), 560–563. https://doi.org/10.1093/bioinformatics/17.6.560

Wu, S. J., Ding, L., & Zhu, J. K. (1996). SOS1, a genetic locus essential for salt tolerance and potassium acquisition. The Plant Cell, 8(4), 617–627.

Xue, B., Zhang, A., & Jiang, M. (2009). Involvement of Polyamine Oxidase in Abscisic Acid-induced Cytosolic Antioxidant Defense in Leaves of Maize. Journal of Integrative Plant Biology, 51(3), 225–234.

Yamasaki, S., & Dillemburg, L. R. (1999). Measurements of leaf relative water content in Araucaria angustifolia. Revista Brasileira de Fisiologia Vegetal, 11, 69–75.

Yang, Q., Chen, Z. Z., Zhou, X. F., Yin, H. B., Li, X., Xin, X. F., Hong, X. H., Zhu, J. K., & Gong, Z. (2009). Overexpression of SOS (Salt Overly Sensitive) genes increases salt tolerance in transgenic Arabidopsis. Molecular Plant, 2(1), 22–31. https://doi.org/10.1093/mp/ssn058

Yildiz, K., Uzal, Ö., & Yilmaz, H. (2008). Consequences of NaCl salinity on growth and ion accumulation in selected strawberry cultivars. European Journal of Horticultural Science, 73(2), 69.

Zhang, H. X., & Blumwald, E. (2001). Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit. Nature Biotechnology, 19(8), 765–768.

Zhang, H. X., Hodson, J. N., Williams, J. P., & Blumwald, E. (2001). Engineering salt-tolerant Brassica plants: Characterization of yield and seed oil quality in transgenic plants with increased vacuolar sodium accumulation. Proceedings of the National Academy of Sciences, 98(22), 12832–12836.

Zhou, F., Mosher, S., Tian, M., Sassi, G., Parker, J., & Klessig, D. F. (2008). The Arabidopsis gain-of-function mutant ssi4 requires RAR1 and SGT1b differentially for defense activation and morphological alterations. Molecular Plant-Microbe Interactions, 21(1), 40–49.

Zhu, J. K., Liu, J., & Xiong, L. (1998). Genetic analysis of salt tolerance in Arabidopsis: Evidence for a critical role of potassium nutrition. The Plant Cell Online, 10(7), 1181–1191.